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Soil dynamic alterations and use efficiency of nitrogen by *Brachiaria* species

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Abstract

Under certain circumstances species of the *Brachiaria* genus, particularly *B. humidicola* might be able to suppress the biological nitrification in soil. This study aimed to investigate the ability of four *Brachiaria* species cultivated in Brazil to promote changes in the N dynamics in the soil as well as its capacity in use efficiently N under low N availability. In a greenhouse condition and using a Quartzipsamment soil, four species of *Brachiaria* (*B. brizantha*, *B. decumbens*, *B. humidicola* and *B. ruziziensis*) and two cut managements ("single cut" – performed at 140 days after seeding (DAS) and "cut/regrowth"– performed at 55 DAS and 85 days after the first cut (140 DAS)), plus a control treatment containing only soil were compared in a factorial design (4×2+1). The following evaluations were performed in plants: dry matter of shoots and roots, total N content, N accumulated and N use efficiency; and soil (which was split in rhizospheric and non-rhizospheric): ammonium, nitrate and total–N contents and pH value. Based on the soil inorganic–N content, there was no detectable effect of species in the soil nitrification process. Moreover, the highest ability of uptaking and using the N was observed in *B. humidicola*, which in the "single cut" management proved to be able to produce 201 g of shoot dry matter in response to each 1 g of N accumulated.

Keywords: *Brachiaria brizantha*; *Brachiaria decumbens; Brachiaria humidicola; Brachiaria ruziziensis*; Nitrification.
Abbreviations: DAS_Days after seeding; DM _Dry Matter; NH_4^+_Ammonium; NO_3^+_nitrate; NUE_Nitrogen Use Efficiency.

Introduction

The importance of *Brachiaria* genus species is due to their adaptability to acid and poor fertile soils, elevated capacity of dry matter production and nutritional quality (Euclides et al., 2009; Timossi et al., 2007). These grasses are largely cultivated on several production systems, for animal feeding or as suppliers of straw to the system. The characteristics related to drought tolerance have made these species important options as coverage plants on no-till systems, especially on areas of dry winter, playing an important role as soil protectors and in nutrient recycling (Borghi and Crusciol, 2007). Some studies suggest that *Brachiaria* genus species have the ability of suppressing the biological soil nitrification (Ishikawa et al., 2003).

The nitrification is a process on which the nitrifying bacteria present on the soil act on the oxidation of ammonium (NH_4^+) in nitrate (NO_3^+). This transformation has a key role on the N cycle, once it dictates the amount of NO_3^- present on the soil and consequently how much NO_3^- can be uptaken by plants or lost via leaching and denitrification (Subbarao et al., 2006). Some plants, especially from the *Brachiaria* genus, seem to be able of producing and releasing some soil chemical substances capable of inhibit the biological nitrification. On a comparative study of 18 species, among forages, cereals and legumes, the highest potential of nitrification inhibition was evidenced to *B. humidicola* and *B. decumbens* (Subbarao et al., 2007).

Subbarao et al. (2009) isolated the main nitrification inhibitor compound from *B. humidicola* roots, as well as they explained its inhibition mechanism. The compound has been called "Brachialactone", acts as inhibitor of *Nitrossomonas* action and is responsible for 60 to 90% of the suppressor activities exercised by the *B. humidicola*. During the oxidation of NH_4^+ to NO_2^- (nitrite) the formation of at least one intermediary compound, the hydroxylamine (NH_2OH) is occurred. The oxidation of NH_4^+ to NH_2OH is mediated by the ammonia monooxygenase enzyme (AMO) and of the NH_2OH to NO_2^- by the hydroxylamine oxidoreductase enzyme (HAO). The "Brachialactone" acts precisely inhibiting the enzymatic AMO and HAO, being this effect higher on the AMO.

The strategy of minimizing the nitrification and so reducing the N losses is necessary both in agriculture and on pasture areas to maintain the N in the NH_4^+ form for more time to synchronize its availability and the plant demands (Ishikawa et al., 2003). The nitrification inhibition with the N maintenance on the NH_4^+ form might be a strategy to prolong the residual time of N in soil as well as to increase this nutrient recuperation on the system and its efficient utilization (Rodgers, 1986).

Plants with higher N use efficiency are important for tropical conditions, on which the N is the main limiting factor of production. Besides, on environments with low N availability, the nitrification inhibition capacity could favor the more efficient use of this element. Thus, this study aimed to investigate the ability of four *Brachiaria* species in altering the dynamic of the N present on the soil over low availability of this element, as well as to understand if such effect can influence the N use by plants under two cutting managements.

Results and Discussion

Shoot dry matter, N accumulation, N use efficiency and N content in soil at 55 days after the seeding (first cut of plants from "cut/regrowth" management)

Among the "two cuts" managed plants we observed that the *Brachiaria* species did not differ only as to the dry matter (DM) of the aerial part, at 55 days after the seeding (DAS) (Table 1). In spite of being more efficient in absorbing and cumulating nitrogen (N), *B. humidicola* showed as low efficient specie in converting the absorbed N into dry matter, producing 78.6 g of DM of aerial part to each g of cumulated N (Table 2). This result, however, can be simply due to the higher N absorption by the *B. humidicola*, once this specie produced the same aerial part DM quantity of the other species.

A different result in terms of DM production of the shoots was observed by Castoldi et al. (2014) in a soil with high N availability, where the DM was higher for *B. ruziziensis* (55.2 g pot^{-1}), followed by *B. decumbens* (27.2 g pot^{-1}), *B. brizantha* (23.7 g pot^{-1}) and *B. Humidicola* (23.5 g pot^{-1}). In this way, it is understood that the N use efficiency by B. Humidicola is increased in soils with low fertility, likely due to the stimulus for production and release of "Brachialactone" compounds (Subbarao et al., 2009), which provides conditions for this species to produce similar values of DM compared to the other species of *Brachiaria*.

On the soil, the NH_4^+, NO_3^- and total–N contents and pH value showed differences at 55 DAS (Table 3). The differences regarding to the NH_4^+ and NO_3^- occurred in relation to the control treatment, in which the *Brachiaria* species cultivation resulted in similar contents of inorganic–N on the soil, but minor in relation to the control (Table 4). As the soil of the not-cultivated control treatment was corrected and fertilized similar to others treatments, the N dynamics led it to cumulate itself in inorganic forms (Cantarella, 2007).

The *B. decumbens* and *B. humidicola* species resulted in higher contents of total–N than the other species to the soil (Table 4). Although is unlikely, this could be related to the finding that these two species have a high capacity to inhibit the nitrification process (Subbarao et al., 2007), which could result in a better protection of the N in soil. Regarding the pH of the soil, the highest value was found on control treatment (Table 4). The lower values of pH in the soils cultivated with brachiarias is likely a result of the absorption process of cations by the plants, which increases the concentration of H^+ ions in the soil due to charge balance, which consequently reduces pH (Marschner, 1995).

Shoot dry matter, accumulated N and N use efficiency at 85 days after the first cut (regrowth phase of plants from "cut/regrowth" management)

On the second cut of the "cut/regrowth" management, differences were observed among the species in reference to the DM, the N-total content and to the N accumulated on shoot and to the N use efficiency (Table 5). The N content considered suitable for *Brachiaria* species ranged from 12 to 20 kg ha^{-1} (Raij et al. 1996). Therefore, all species presented low N contents in shoots (Table 6), which indicates that the soil and/or the fertilization was not enough to promote appropriate N supply to the plants. The minor content among the species, presented by *B. humidicola*, probably occurred due to the dilution effect (Lemaire and Chartier, 1992), since this species was the one that produced more DM shoots from the first cut, which was the most efficient in using N.

The remobilization effect from the reservations to the regrowth process of the plants might have led the plants to the stress situation (Sbrissia et al., 2007). Among the species, the *B. humidicola* was the one that better responded to that situation, represented by the highest value of N use efficiency. The N accumulation on the aerial part of the plants is an important factor to be considered once it plays a fundamental role on the N cycling on the soil-plant system. The withheld N on the straw works as N reservation, since part of it will return to the soil after the straw mineralization (Rosolem et al., 2010).

Shoot and root dry matter, N accumulation, N use efficiency and Ncontent in soil at 140 days after the seeding (of plants from both cut managements)

Regarding to the variables related to the aerial part of the plants and for both managements, the interaction "species × cut managements" was significant to DM, N accumulation and N use efficiency (Table 7). For the total–N content, the effect occurred singly to each of the factors. So the plants subjected to the "cut/regrowth" management presented higher N total content in shoots (6.66 g kg^{-1}) than the ones that were cut only at the end of the study ("single cut" management) (5.88 g kg^{-1}). This fact is likely a consequence of the higher shoot DM of the plants subjected to the "cut/regrowth" management, particularly the *B. humidicola* (Table 8). Among the species, the lowest total–N content was found on the aerial part of the *B humidicola* (5.39 g kg^{-1}). *B. brizantha, B. decumbens* and *B. ruziziensis* presented a total–N content of 6.46, 6.46 and 6.77 g kg^{-1}, respectively. It is important to highlight that these contents are low (Raij et al., 1996) even lower than those found at the 55 DAS. The evidences demonstrated that after 140 days of cultivation the N would be limiting the plants development even more.

The DM production of *B. brizantha* and *B. decumbens* was not affected by the cut management (Table 7). However, *B. humidicola* and *B. ruziziensis* produced more shoot DM when submitted to two cuts (Table 8). When cut only at the 140 DAS ("single cut"), the *B. decumbens* presented higher DM compared to the *B. brizantha* and to the *B. ruziziensis* (Table 8). All the species accumulated higher amounts of N in aerial parts when subjeced to the "cut/regrowth" management (Table 8). This result is probably due to the plants response to the cut process, by delaying the maturation phase and stimulating the plants to maintain young structures and high nutrient contents for a little bit longer (Paulino et al., 2001).

Between the managements, *B. humidicola* has been one of the species that mostly accumulated N in the aerial part when managed in "cut/regrowth", and one of those with less N accumulation when cut only at the end of the study (Table 8). The cut performed at 55 DAS seems to have stimulated the *B. humidicola* in higher proportion than the other species, once it presented increment on the DM production absorbing a similar N amount than the others.

The highest N uptake by the plants managed under "cut/regrowth", however, was not followed in the same proportion by the DM production, which resulted in lower efficiency in N use (Table 8). This result might be related to the cutting process which prolongs the vegetative period and stimulates the plant to maintain itself with elevated contents of nutrients for a longer time. This could have resulted in higher N uptake (Paulino et al., 2001).

Table 1. Value and significance of F and coefficient of variation (CV) for the variables dry matter (DM), total nitrogen content (Total–N), nitrogen accumulated in the aerial part (N accumulated) and nitrogen use efficiency (NUE) by species of brachiaria, 55 days after seeding.

	DM	Total–N	N accumulated	NUE
Species	0.539^{ns}	3.890*	2.671*	4.607*
CV (%)	11.1	11.8	10.6	8.51

^{ns}, * and **: F not significant, significant at 5% and significant at 1%, respectively.

Table 2. Dry matter (DM), total nitrogen content (Total–N) and nitrogen accumulation in aerial parts, and nitrogen use efficiency (NUE) by species of brachiaria, 55 days after sowing.

Species	DM	Total–N	N accumulated	NUE
	g pot^{-1}	g kg^{-1}	g pot^{-1}	g DM g^{-1} N accum
B. brizantha	22.0	10.7 b	0.23 b	95.7 a
B. decumbens	21.5	11.2 b	0.24 b	89.6 a
B. humidicola	21.2	13.0 a	0.27 a	78.6 b
B. ruziziensis	22.8	10.7 b	0.24 ab	95.0 a

Means followed by different letters, in the column and for each variable, differ by t test (LSD) at 5% probability.

Table 3. Value and significance of F and coefficient of variation (CV) for the variables ammonium content (NH_4^+), nitrate content (NO_3^-), total nitrogen content (Total–N) and pH of soil uncultivated or cultivated with brachiarias, 55 days after seeding.

	NH_4^+	NO_3^-	Total–N	pH
Species (+ control)	2.918*	2.413*	3.869*	3.418*
CV (%)	51.6	43.1	2.76	1.72

^{ns}, * and **: F not significant, significant at 5% and significant at 1%, respectively.

Table 4. Inorganic nitrogen contents (NH_4^+ and NO_3^-), total nitrogen (Total–N) and pH in a Quartzipsamment soil uncultivated or cultivated with species of *Brachiaria*, 55 days after seeding.

Espécies	NH_4^+	NO_3^-	Total–N	pH
	------------- mg dm^{-3} -------------			
B. brizantha	1.6 b	2.5 b	411 b	4.6 ab
B. decumbens	2.4 b	2.7 b	436 a	4.9 b
B. humidicola	2.9 b	3.0 b	428 ab	4.5 b
B. ruziziensis	2.9 b	2.8 b	417 b	4.6 ab
Control	5.1 a	5.2 a	411 b	4.7 a

Means followed by different letters, in the column and for each variable, differ by t test (LSD) at 5% probability.

Table 5. Value and significance of F and coefficient of variation (CV) for the variables dry matter (DM), total nitrogen content (Total–N), nitrogen accumulated in the aerial part (N accumulated) and nitrogen use efficiency (NUE) by species of brachiaria in the regrowth phase (85 days after first cut).

	DM	Total–N	N accumulated	NUE
Species	11.385**	5.767**	1.847*	6.833**
CV (%)	11.6	9.22	13.2	9.29

^{ns}, * and **: F not significant, significant at 5% and significant at 1%, respectively.

Table 6. Dry matter (DM), total nitrogen content (total–N) and nitrogen accumulated in the aerial part, and nitrogen use efficiency (NUE) by species of brachiarias in the regrowth phase (85 days after first cut).

Species	DM	Total–N	N accumulated	NUE
	g pot^{-1}	g kg^{-1}	g pot^{-1}	g DM g^{-1} N accum
B. brizantha	31.5 b	6.83 a	0.21 b	150.0 b
B. decumbens	34.2 b	7.01 a	0.24 ab	142.5 b
B. humidicola	44.5 a	5.77 b	0.26 a	171.2 a
B. ruziziensis	34.0 b	7.03 a	0.25 ab	136.0 b

Means followed by different letters, in the column and for each variable, differ by t test (LSD) at 5% probability.

Table 7. Value and significance of F and coefficient of variation (CV) for the variables dry matter (DM), total nitrogen content (Tot–N), nitrogen accumulated (N ac) and nitrogen use efficiency (NUE) in the aerial part; and DM, Tot–N, N ac, total length (TL), surface area (SA) and mean diameter (Ø) by species of brachiaria, 140 days after seeding.

	------------- Aerial part -------------				----------------------------- Roots -----------------------------					
	DM	Tot–N	N ac	NUE	DM	Tot–N	N ac	TL	SA	Ø
Species	5.64**	12.9**	2.090^{ns}	9.33**	2.319^{ns}	3.68**	3.494*	2.829^{ns}	2.369^{ns}	3.043^{ns}
Managem.	1.785^{ns}	21.4**	207**	152**	14.1**	4.267*	27.3**	1.167^{ns}	2.402^{ns}	0.201^{ns}
Specie x Managem.	4.112*	0.487^{ns}	5.72**	4.227*	0.222^{ns}	1.478^{ns}	1.119^{ns}	0.229^{ns}	0.156^{ns}	0.917^{ns}
CV (%)	9.10	9.27	9.26	10.2	20.2	26.3	23.7	28.7	24.4	6.91

^{ns}, * and **: F not significant, significant at 5% and significant at 1%, respectively.

Table 8. Dry matter (DM), nitrogen accumulated in the aerial part and nitrogen use efficiency (NUE) of *B. brizantha*, *B. decumbens*, *B. humidicola* and *B. ruziziensis*, 140 days after seeding and as affected by cut management ("Cut/Regrowth" vs. "Single cut").

Species	Cut Management	
	Cut/Regrowth	Single cut
	--------------------- DM (g pot^{-1}) -------------------	
B. brizantha	53.3 bA[1]	52.0 bA
B. decumbens	55.8 bA	61.0 aA
B. humidicola	65.8 aA	56.2 abB
B. ruziziensis	57.0 bA	54.8 bB
	---------------- Accumulated N (g pot^{-1}) ----------------	
B. brizantha	0.47 abA	0.32 bcB
B. decumbens	0.50 abA	0.36 aB
B. humidicola	0.51 aA	0.28 cB
B. ruziziensis	0.46 bA	0.35 abB
	---------------- NUE (g DM/g N accum) ---------------	
B. brizantha	113.4 aB	162.5 bA
B. decumbens	111.6 aB	169.4 bA
B. humidicola	129.0 aB	200.7 aA
B. ruziziensis	123.9 aB	156.6 bA

[1]Sum of the amounts of DM produced at 55 days after sowing (DAS) and 85 days after the first cut (140 DAS).
Means followed by different lowercase letters in the column, and different capitals Letter in the row, and for each variable, differ by t test (LSD) at 5% probability.

Table 9. Total nitrogen content (Total–N) and nitrogen accumulated in roots of *B. brizantha*, *B. decumbens*, *B. humidicola* and *B. ruziziensis*, 140 days after seeding.

Species	Total–N	N accumulated
	g kg^{-1}	g pot^{-1}
B. brizantha	7.33 ab	0.37 a
B. decumbens	6.94 b	0.30 ab
B. humidicola	9.15 a	0.37 a
B. ruziziensis	6.02 b	0.27 b

Means followed by different letters, in the column and for each variable, differ by t test (LSD) at 5% probability.

Table 10. Value and significance of F and coefficient of variation (CV) for the variables ammonium content (NH_4^+), nitrate content (NO_3^-), total nitrogen content (Total–N) and pH in soil (rizospheric and non rizospheric) cultivated with species of brachiaria under different cut management, 140 days after seeding.

	NH_4^+	NO_3^-	N-total	pH
Species	4.213*	4.060*	0.901^{ns}	1.949^{ns}
Management	8.115**	0.918^{ns}	0.278^{ns}	0.006^{ns}
Soil	0.037^{ns}	0.104^{ns}	3.630^{ns}	81.53**
Specie x Management	3.080*	1.993^{ns}	2.806*	0.387^{ns}
Specie x Soil	2.134^{ns}	1.374^{ns}	1.120^{ns}	3.096*
Management x Soil	0.049^{ns}	0.884^{ns}	3.162^{ns}	4.334*
Specie x Manag x Soil	0.139^{ns}	1.331^{ns}	0.377^{ns}	1.232^{ns}
Factorial x Control	5.459*	192.1**	0.412^{ns}	22.98**
CV (%)	36.9	31.7	8.74	2.34

^{ns}, * and **: F not significant, significant at 5% and significant at 1%, respectively.

Table 11. Ammonium content (NH_4^+) and total nitrogen content (Total–N) in soil cultivated with *B. brizantha*, *B. decumbens*, *B. humidicola* and *B. ruziziensis*, 140 days after soeeding and as affected by management ("Cut/Regrowth" or "Single cut").

Species	Management	
	"Cut/Regrowth"	"Single cut"
	-------------------- NH_4^+ (mg dm^{-3}) --------------------	
B. brizantha	3.5 abA	3.6 cA
B. decumbens	4.7 aA	5.2 abA
B. humidicola	3.0 bA	3.8 bcA
B. ruziziensis	3.2 abB	6.4 aA
	------------------- Total–N (mg dm^{-3}) -------------------	
B. brizantha	436 aA	401 bA
B. decumbens	429 aA	407 bA
B. humidicola	407 aA	409 bA
B. ruziziensis	411 aA	446 aA

Means followed by different lowercase letters in the column, and diferente capitals Letter in the row, and for each variable, differ by t test (LSD) at 5% probability.

A high N accumulation by itself is already a desirable characteristic, once the N on the plant remains protected of loss processes and might be more efficiently recycled on the system. This may reduce the production costs, avoid natural resources degradation and increase the crops yield (Costa et al., 2009). Besides, the N has an important function in structural characteristics of the plants, which could positively influence the *Brachiaria*'s production (Silva et al., 2013) and improve the management when this forage is used to animal production.

As already mentioned, the N use efficiency was higher on the "single cut" management, regardless of the specie (Table 8). However, special attention must be given to the higher N use efficiency by the *B. humidicola*, which presented the capacity of produce 201 g of DM to each g of accumulated N in the aerial part. Meanwhile, the other species presented an average capacity of 163 g of DM to each g of N g (Table 8). This result evidences the higher capacity of the *B. humidicola* in uptaking and using the N in low availability conditions, as we observed in the present study.

When the variables related to the roots were compared, the interaction "species × cut managements" was not significant. Among the species, differences related to the total–N content and N accumulated on the roots were observed (Table 9). The higher total–N contents on the roots were found on the *B. brizantha* and *B. humidicola*. This fact explains the higher amount of accumulated N on the roots of these species (Table 9). The accumulated N on the roots and aerial parts can be related to the plant's capacity in uptaking and accumulating N with important implications on the inclusion of the species in production systems. The total length, superficial area and roots average diameter were not differed among the species, as well as they were not affected by the managements. The total length average values, the superficial area and the roots average diameter were 95.02 cm, 13.25 cm^2 and 0.46 mm, respectively.

The DM (52.1 g pot^{-1}), as the amount of N accumulated on the roots (0.40 g $vase^{-1}$) were higher on the plants cut only at the 140 DAS. The "cut/regrowth" management resulted in reduction of 24% of the root DM and lower N accumulation (0.25 g pot^{-1}). This result is probably attributed to the aerial part cut, which could have provoked greater root death or even N remobilization to the process of regrowth.

The interaction "species × management × soil (rizospheric or non rizospheric)" was not significant in any of analyzed variables (Table 10). However, there was significant "specie × management" interaction to the NH_4^+ and total–N contents (Table 10). The NO_3^- content was singly affected by the species (Table 10). The pH value was affected as function of interactions "species × soil" and "management × soil" (Table 10).

Differences between the managements for NH_4^+ on the soil were only found in *B. ruziziensis*, when one single cut time left the NH_4^+ content approximately twice times higher when it was cut twice (Table 11). Among the species, it was not possible to found a pattern to the NH_4^+ content on the soil (Table 11). So that, the higher NH_4^+ contents were found on the soil cultivated with *B. decumbens* ("cut/regrowth") and *B. ruziziensis* ("single cut"), which suggests that these species are equally and more capable of inhibiting the biological nitrification on the soil than the *B. humidicola*, as reference of nitrification suppressor plant (Subbarao et al., 2009). The intensity of the suppressor effect depends on various factors such as humidity and soil temperature (Ipinmoroti et al., 2008), and nutritional state of the plant (Subbarao et al., 2006). In addition, if that capacity presents, it would also grant lower NO_3^- contents to the soil cultivated with these species. The highest NO_3^- contents were found in the soils cultivated with *B. decumbens* and *B. ruziziensis* (4.6 and 4.9 mg dm^{-3}, respectively). The lowest contents were found at the *B. brizantha* (3.7 mg dm^{-3}) and *B. humidicola* (3.3 mg dm^{-3}). These results could have occurred because of differences related to the preferential form of absorption and to the amount of N uptaken by the species, or even effects of these species on the nitrification process. However, these differences are not enough to justify or characterize the inhibiting nitrification effect.

Another fact that must be considered is that, if inhibition effect has occurred, it might be in a reduced scale which would not justify the cultivation of any of these species aiming only to reduce losses and/or to improve N use efficiency on the system. In fact, these results confirm the observation made by Castoldi et al. (2013), where only the variances observed on the NH_4^+ and NO_3^- contents on the soil. Therefore, it was not enough to confirm the nitrification suppressor effect of the *B. humidicola, B. brizantha, B. decumbens* and *B. ruziziensis*. The authors attributed the effect to the conditions of high N availability.

The average NO_3^- value that found on the soil cultivated with the *Brachiaria* (4.1 mg dm^{-3}) was lower compared to the control soil (14.9 mg dm^{-3}). Again, it is due to the fact that control soil has not been cultivated, resulted in N accumulation in NO_3^- form. The absence of differences – to the inorganic–N content – corroborates the Herman et al. (2006) results, who observed similar nitrification rates on rizhospheric and non-rhizospheric soils cultivated with *Avena bartata*, a common grass in North American forests.

Although the total–N content has been affected by the interaction "species × management", no difference was found between the managements (Table 10). Among the species, the difference has occurred when they were cut single time, with higher total N content in soils cultivated with *B. ruziziensis* (Table 11). This specie could have been the one that provided the fastest decomposition of the roots or even higher exudation of N compounds by the active roots.

Regarding to the soil pH, independent from species or the cut management, the values found on the rhizospher (4.1) were always lower than those found on the non-rhizospheric soil (4.4). This represents that acidification effect occurs in the root zone of the plants (Andrade and Nogueira, 2005). Likely as occurred at 55 DAS (Table 1), the pH value of the control soil (4.3) was higher than those found on the soil cultivated with *Brachiaria* species (4.0).

Overall, it was not possible to detect the species (*B. brizantha, B. decumbens, B. humidicola and B. ruziziensis*) effect on the soil nitrification process. However, we demonstrated that *B. humidicola* is more capable of uptaking and using the N efficiently in low conditions of N availability.

Materials and Methods

Site and soil

The experiment was conducted under greenhouse conditions, in Botucatu, SP, Brazil. The soil classified as Quartzipsamment soil (Embrapa, 2006) presented 105 g kg^{-1} of clay, 41 g kg^{-1} of silt and 854 g kg^{-1} of sand. On the chemical analysis (Raij et al., 2001) the following results were found: pH ($CaCl_2$) of 5.4, 12 g dm^{-3} of organic matter, 3 mg dm^{-3} of phosphorus (P) (resin), 0.9 $mmol_c$ dm^{-3} of potassium (K), 23 $mmol_c$ dm^{-3} of calcium (Ca), 9 $mmol_c$ dm^{-3} of magnesium (Mg), sum of bases of 32.9 $mmol_c dm^{-3}$, CTC of 53 $mmol_c$ dm^{-3}, bases saturation as 60% and 400 mg dm^{-3}

of total–N, determined according method described by Bremmer and Mulvaney (1982). The plants were cultivated in ceramic pots wrapped in aluminum paper and filled with 10 dm^{-3} of dry soil sifted in a 2 mm mesh sieve. Prior to the experiment implementation the soil was mixed with 100, 150, and 120 mg dm^{-3} of N, P, and K, as ammonium sulfate, triple superphosphate and potassium chloride, respectively.

Treatments and plant material

The treatments were composed of four species of *Brachiaria* (*B. brizantha* cv. Marandú, *B. decumbens*, *B. humidicola* and *B. ruziziensis*) and of two cutting management ("single cut" – performed at 140 days after seeding (DAS) and "cut/regrowth"– performed at 55 DAS and 85 days after the first cut (140 DAS)) plus a control treatment (containing only soil), with six replicates, totalizing 52 pots. The handling strategies were adopted aiming to evaluate if the plant reservation mobilization to the regrowth has affected the N soil dynamics.

Experimental conduction and measurements

The seeds were pre-germinated in gerbox® boxes at 25 °C during 60 hours. After pre-germination, 24 seeds were seeded in each pot. The thinning was performed at 10 DAS, when six plants per pot were left. The pots were daily irrigated aiming to maintain the soil moisture near to 70% of the field capacity.

At the 55 DAS, the plants of the "cut/regrowth" management were cut at 10 cm of the soil surface. The material (stems and leaves) was washed in distillated water and dried in a drying oven (with forced air circulation), at 65 °C until reached a constant weigh. Then, it was weighed, milled in a Willey type mill and analyzed to measure the total–N content (AOAC, 1995). The N use efficiency was calculated by dividing the dry matter of the aerial part (DM) (g pot^{-1}) per the total amount of N accumulated on it (g pot^{-1}). At the 55 DAS, soil samples were also taken for determination of the total–N, NH_4^+ and NO_3^- content and pH value. The soil was taken with an auger (probe type) – with 2.5 cm of diameter – until 10 cm depth, aiming to protect the plant roots from damage. To preserve the NH_4^+ and the NO_3^- contents, the samples were put in plastic bags and conditioned in a freezer at -5 °C. The NH_4^+ and NO_3^- content determination was performed via potassium chloride extraction (KCl) and posterior distillation (Keeney and Nelson, 1982). The total–N content was determined by sulfuric digestion and posterior distillation (Bremmer and Mulaney, 1982) and the pH by $CaCl_2$ extraction (Raij et al., 2001).

At the 140 DAS, all the plants were cut from the soil surface and submitted to the same procedures as used for the plants from 55 DAS sampling. The dry matter accumulated until 140 DAS was calculated – DM of the "cut/regrowth" management was represented by sum of the DM produced at the 55 DAS and at 85th day after the first cut. The N use efficiency was calculated as previously described.

The soil, still with the root system, was transferred to a bowl and gently agitated, being considered as rhizospheric soil the one that stayed adhered to the roots (Marilley et al., 1998). The non-rhizospheric soil (that did not adhere to the roots) was homogenized and a sample was collected and sifted in a 2 mm mesh sieve. The rhizospheric soil was all sifted due to the little portion obtained of it. Both of the soil samples, once sifted, were divided in two samples: one that was put immediately in the freezer at -5 °C to preserve the inorganic–N content and other one that was dried on air for total–N and pH analysis.

The roots were manually separated from the rhizospheric soil portion and posteriorly washed. Sub-samples of the roots were collected and conditioned in alcoholic solution at 70% to posterior evaluation of length, medium diameter and superficial area. Those analyses were performed in a scanner attached to a computer containing the WinRhizo Software. The other roots were conditioned in paper bags, dried in a drying oven (with forced air circulation) at 65 °C until reached a constant weigh and so, weighted.

The samples used in the WinRhizo analysis were also dried, and its weigh was summed up to the remainder of the sample which was milled and analyzed with regard to the total–N content (AOAC, 1995) and N accumulated.

Experimental design and statistical analysis

The experiment design utilized was a randomized block with six replicates. The analysis was performed only with the "cut/regrowth" management (55 DAS and 85 days after the first cut – which characterized the regrowth phase). The data were submitted to variance analysis and the species were compared to the average t test (LSD, $p<0.05$), using the SISVAR statistic program (Ferreira, 2000).

To the variables related to the final evaluation of the plants (140 DAS), the experimental design was a factorial 4×2 (four species and two managements). The results were submitted to analysis of variance and means comparison by t test (LSD, $p < 0.05$), using the SISVAR statistic program (Ferreira, 2000). In turn, the results obtained from the soil were analyzed in a factorial scheme 4×2×2+1 (four species, two managements and two soil portion – rhizospheric and non-rhizospheric, plus a control treatment), utilizing the ASSISTAT statistic program (Silva and Azevedo, 2002), and likewise were submitted to analysis of variance and means comparison by t test (LSD, $p < 0.05$).

Conclusion

No soil nitrification was detected in species (*B. brizantha, B. decumbens, B. humidicola* and *B. ruziziensis*). Performing two cuts – compared to one single cut – increased the N uptake and accumulation on the *B. brizantha, B. decumbens, B. humidicola* and *B. ruziziensis* species, which also increased the dry matter production. The *B. humidicola* showed itself as more capable of uptaking and efficiently using the N under low N availability conditions.

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